

WHAT IS CLAIMED IS:

1. ICAM-1, or a functional derivative thereof, substantially free of natural contaminants.

2. The ICAM-1 of claim 1, wherein said ICAM-1 is additionally capable of binding to a molecule present on the surface of a lymphocyte.

3. The ICAM-1 molecule of claim 2, wherein said molecule additionally contains at least one polypeptide selected from the group consisting of:

- (a) -V-T-C-S-T-S-C-D-Q-P-K;
- (b) -X-G-S-V-L-V-T-C-S-T-S-C-D-Q-P-K;
- (c) -L-L-G-I-E-T-P-L;
- (d) -F-L-T-V-Y-X-T;
- (e) -V-E-L-A-P-L-P;
- (f) -E-L-D-L-R-P-Q-G-L-E-L-F-E;
- (g) -L-N-P-T-V-T-Y-G-X-D-S-F-S-A-K;
- (h) -S-F-P-A-P-N-V;
- (i) -L-R-G-E-K-E-L;
- (j) -R-G-E-K-E-L-K-R-E-P;
- (k) -L-R-G-E-K-E-L-K-R-E-P-A-V-G-E-P-A-E;
- (l) -P-R-G-G-S;
- (m) -P-G-N-N-R-K;
- (n) -Q-E-D-S-Q-P-M;
- (o) -T-P-E-R-V-E-L-A-P-L-P-S;
- (p) -R-R-D-H-H-G-A-N-F-S; and
- (q) -D-L-R-P-Q-G-L-E.

4. A functional derivative of the ICAM-1 of claim 2 wherein said functional derivative is a fragment of ICAM-1 which is capable of binding to a molecule present on the surface of a lymphocyte.

5. A functional derivative of the ICAM-1 of claim 2 wherein said functional derivative is a variant of ICAM-1 which is capable of binding to a molecule present on the surface of a lymphocyte.

6. A functional derivative of the ICAM-1 of claim 2 wherein said functional derivative is an analog of ICAM-1 which is capable of binding to a molecule present on the surface of a lymphocyte.

7. A functional derivative of the ICAM-1 of claim 2 wherein said functional derivative is a chemical derivative of ICAM-1 which is capable of binding to a molecule present on the surface of a lymphocyte.

8. A recombinant DNA molecule capable of expressing ICAM-1 or a functional derivative thereof.

9. The DNA molecule of claim 8, wherein said ICAM-1 or said functional derivative thereof is capable of encoding at least one polypeptide selected from the group consisting of:

- (a) -V-T-C-S-T-S-C-D-Q-P-K;
- (b) -X-G-S-V-L-V-T-C-S-T-S-C-D-Q-P-K;
- (c) -L-L-G-I-E-T-P-L;
- (d) -F-L-T-V-Y-X-T;
- (e) -V-E-L-A-P-L-P;
- (f) -E-L-D-L-R-P-Q-G-L-E-L-F-E;
- (g) -L-N-P-T-V-T-Y-G-X-D-S-F-S-A-K;
- (h) -S-F-P-A-P-N-V;
- (i) -L-R-G-E-K-E-L;
- (j) -R-G-E-K-E-L-K-R-E-P;
- (k) -L-R-G-E-K-E-L-K-R-E-P-A-V-G-E-P-A-E;
- (l) -P-R-G-G-S;
- (m) -P-G-N-N-R-K;

- (n) -Q-E-D-S-Q-P-M;
- (o) -T-P-E-R-V-E-L-A-P-L-P-S;
- (p) -R-R-D-H-H-G-A-N-F-S; and
- (q) -D-L-R-P-Q-G-L-E.

10. A method for recovering ICAM-1 in substantially pure form which comprises the steps:

- (a) solubilizing ICAM-1 from the membranes of cells expressing ICAM-1, to form a solubilized ICAM-1 preparation,
- (b) introducing said solubilized ICAM-1 preparation to an affinity matrix, said matrix containing immobilized antibody capable of binding to ICAM-1,
- (c) permitting said ICAM-1 to bind to said antibody of said affinity matrix,
- (d) removing from said matrix any compound incapable of binding to said antibody and
- (e) recovering said ICAM-1 in substantially pure form by eluting said ICAM-1 from said matrix.

11. The method of claim 10 wherein said method additionally comprises the steps:

- (f) purifying said recovered ICAM-1 of step (e) by preparative gel electrophoresis, and
- (g) eluting said recovered ICAM-1 from a gel employed in step (f).

12. The ICAM-1 produced by the method of any one of claims 10-11.

13. An antibody capable of binding to a molecule selected from the group consisting of ICAM-1, and a functional derivative of ICAM-1.

14. The antibody of claim 13, wherein said antibody is a monoclonal antibody.

15. The monoclonal antibody of claim 14, which is R6-5-D6.

16. The antibody of claim 13 wherein said molecule is capable of binding to a receptor present on the surface of a lymphocyte, and wherein the binding of said antibody to said molecule impairs the ability of said molecule to bind to said receptor molecule of said lymphocyte.

17. The antibody of claim 16, wherein said antibody is a monoclonal antibody.

18. The antibody of any one of claims 13-17, in labeled form.

19. A hybridoma cell capable of producing the monoclonal antibody of any one of claims 14, 15, and 17.

20. The hybridoma cell capable of producing the monoclonal antibody R6-5-D6, said cell being ATCC HB 9580.

21. A fragment of the antibody of any one of claims 13-17, said fragment being capable of binding said molecule.

22. A method for producing a desired hybridoma cell that produces an antibody which is capable of binding to ICAM-1, or its functional derivative, which comprises the steps:

- (A) immunizing an animal with a cell expressing ICAM-1,
- (B) fusing the spleen cells of said animal with a myeloma cell line,
- (C) permitting the fused spleen and myeloma cells to form antibody secreting hybridoma cells, and
- (D) screening said hybridoma cells for said desired hybridoma cell that is capable of producing an antibody capable of binding to ICAM-1.

23. The method of claim 22, wherein in step (A) said animal is immunized with a cell expressing ICAM-1 but not expressing LFA-1, and wherein said screening step (D) comprises the steps:

(1) incubating the antibody secreted from any of said hybridoma cells with a lymphocyte preparation, said lymphocyte preparation containing a plurality of cells capable of aggregating,

(2) examining said secreted antibody for the capacity to inhibit the aggregation of said cells of said lymphocyte preparation, and

(3) selecting as said desired hybridoma cell a hybridoma cell that produces an antibody capable of inhibiting said aggregation of said cells of said lymphocyte preparation.

24. The method of claim 22 wherein said screening step (D) comprises the steps:

(1) incubating the antibody secreted from any of said hybridoma cells with: a lymphocyte preparation containing a plurality of cells having the characteristics of:

(a) being capable of aggregating, and

(b) being incapable of aggregating in the presence of an antibody capable of binding ICAM-1,

(2) verifying that said antibody secreted from said hybridoma cell does not bind to a member of the LFA-1 family of molecules,

(3) selecting as said desired hybridoma cell a hybridoma cell that produces an antibody capable of inhibiting the spontaneous aggregation of the cells of said lymphocyte preparation, and

(4) verifying that said antibody selected from said hybridoma cell does not bind to a member of the LFA-1 family of molecules.

25. The hybridoma cell obtained from the method of any one of claims 22-24.

26. The antibody produced by the hybridoma cell of claim 25.

27. A method of identifying a non-immunoglobulin antagonist of intercellular adhesion which comprises:

(A) incubating a non-immunoglobulin agent capable of being an antagonist of intercellular adhesion with a lymphocyte preparation, said lymphocyte preparation containing a plurality of cells capable of aggregating,

(B) examining said lymphocyte preparation to determine whether the presence of said agent inhibits the aggregation of said cells of said lymphocyte preparation; wherein inhibition of said aggregation identifies said agent as an antagonist of intercellular adhesion.

28. A method for treating inflammation resulting from a response of the specific defense system in a mammalian subject which comprises providing to a subject in need of such treatment an amount of an anti-inflammatory agent sufficient to suppress said inflammation; wherein said anti-inflammatory agent is selected from the group consisting of: an antibody capable of binding to ICAM-1; a fragment of an antibody, said fragment being capable of binding to ICAM-1; ICAM-1; a functional derivative of ICAM-1; and a non-immunoglobulin antagonist of ICAM-1.

29. The method of claim 28, wherein said non-immunoglobulin antagonist of ICAM-1 is a non-immunoglobulin antagonist of ICAM-1 other than LFA-1.

30. The method of claim 28, wherein said anti-inflammatory agent is an antibody capable of binding to ICAM-1.

31. The method of claim 30, wherein said antibody is a monoclonal antibody.

32. The method of claim 31, wherein said monoclonal antibody is the monoclonal antibody R6-5-D6.

33. The method of claim 28, wherein said anti-inflammatory agent is a fragment of an antibody, said fragment being capable of binding to ICAM-1.

34. The method of claim 33, wherein said fragment is a fragment of the antibody R6-5-D6.

35. The method of claim 28, wherein said inflammation is a reaction of the specific defense system.

36. The method of claim 28, wherein said inflammation is a delayed type hypersensitivity reaction.

37. The method of claim 28, wherein said inflammation is a symptom of psoriasis.

38. The method of claim 28, wherein said inflammation is a symptom of an autoimmune disease.

39. The method of claim 38, wherein said autoimmune disease is selected from the group consisting of Reynaud's syndrome, autoimmune thyroiditis, EAE, multiple sclerosis, rheumatoid arthritis and lupus erythematosus.

40. The method of claim 28, wherein said inflammation is in response to organ transplant rejection.

41. The method of claim 28, wherein said inflammation is in response to tissue graft rejection.

42. The method of claim 28, which additionally comprises the co-administration of an agent selected from the group consisting of: an antibody capable of binding to LFA-1; a functional derivative of an antibody, said functional derivative being capable of binding to LFA-1; and a non-immunoglobulin antagonist of LFA-1.

43. A method of suppressing the metastasis of a hematopoietic tumor cell, said cell requiring a functional member of the LFA-1 family for migration, which method comprises providing to a patient in need of such treatment an amount of an anti-inflammatory agent sufficient to suppress said metastasis; wherein said anti-inflammatory agent being selected from the group consisting of: an antibody capable of binding to ICAM-1; a fragment of an antibody, said fragment being capable of binding to ICAM-1; ICAM-1; a functional derivative of ICAM-1; and a non-immunoglobulin antagonist of ICAM-1.

44. The method of claim 43, wherein said non-immunoglobulin antagonist of ICAM-1 is a non-immunoglobulin antagonist of ICAM-1 other than LFA-1.

45. The method of claim 43, wherein said anti-inflammatory agent is an antibody capable of binding to ICAM-1.

46. The method of claim 45, wherein said antibody is a monoclonal antibody.

47. The method of claim 46, wherein said monoclonal antibody is the monoclonal antibody R6-5-D6.

48. The method of claim 43, wherein said anti-inflammatory agent is a fragment of an antibody, said fragment being capable of binding to ICAM-1.



49. The method of claim 48, wherein said fragment is a fragment of the antibody R6-5-D6.

50. A method of suppressing the growth of an ICAM-1-expressing tumor cell which comprises providing to a patient in need of such treatment an amount of a toxin sufficient to suppress said growth, said toxin being selected from the group consisting of a toxin-derivatized antibody capable of binding to ICAM-1; a toxin-derivatized fragment of an antibody, said fragment being capable of binding to ICAM-1; a toxin-derivatized member of the LFA-1 family of molecules; and a toxin-derivatized functional derivative of a member of the LFA-1 family of molecules.

51. A method of suppressing the growth of an LFA-1-expressing tumor cell which comprises providing to a patient in need of such treatment an amount of a toxin sufficient to suppress said growth, said toxin being selected from the group consisting of a toxin-derivatized ICAM-1; and a toxin-derivatized functional derivative of ICAM-1.

52. A method of diagnosing the presence and location of inflammation resulting from a response of the specific defense system in a mammalian subject suspected of having said inflammation which comprises:

(a) administering to said subject a composition containing a detectably labeled binding ligand capable of identifying a cell which expresses ICAM-1, and

(b) detecting said binding ligand.

53. A method of diagnosing the presence and location of inflammation resulting from a response of the specific defense system in a mammalian subject suspected of having said inflammation which comprises:

- (a) incubating a sample of tissue of said subject with a composition containing a detectably labeled binding ligand capable of identifying a cell which expresses ICAM-1, and
- (b) detecting said binding ligand.

54. The method of any one of claims 52 or 53 wherein said binding ligand is bound in said sample of said tissue.

55. The method of any one of claims 52 or 53 wherein said binding ligand is capable of binding to ICAM-1, said ligand being selected from the group consisting of an antibody and a fragment of an antibody.

56. The method of claim 55, wherein said antibody is a monoclonal antibody.

57. The method of claim 56, wherein said monoclonal antibody is the monoclonal antibody R6-5-D6.

58. The method of any one of claims 52 or 53 wherein said binding ligand is a nucleic acid molecule capable of binding to a molecule selected from the group consisting of a DNA sequence of ICAM-1, and an mRNA sequence of a gene for ICAM-1.

59. The method of claim 58 wherein said nucleic acid molecule encodes at least one polypeptide selected from the group consisting of:

- (a) -V-T-C-S-T-S-C-D-Q-P-K;
- (b) -X-G-S-V-L-V-T-C-S-T-S-C-D-Q-P-K;
- (c) -L-L-G-I-E-T-P-L;
- (d) -F-L-T-V-Y-X-T;
- (e) -V-E-L-A-P-L-P;
- (f) -E-L-D-L-R-P-Q-G-L-E-L-F-E;

- (g) -L-N-P-T-V-T-Y-G-X-D-S-F-S-A-K;
- (h) -S-F-P-A-P-N-V;
- (i) -L-R-G-E-K-E-L;
- (j) -R-G-E-K-E-L-K-R-E-P;
- (k) -L-R-G-E-K-E-L-K-R-E-P-A-V-G-E-P-A-E;
- (l) -P-R-G-G-S;
- (m) -P-G-N-N-R-K;
- (n) -Q-E-D-S-Q-P-M;
- (o) -T-P-E-R-V-E-L-A-P-L-P-S;
- (p) -R-R-D-H-H-G-A-N-F-S; and
- (q) -D-L-R-P-Q-G-L-E.

60. A method of diagnosing the presence and location of an ICAM-1-expressing tumor cell in a mammalian subject suspected of having such a cell, which comprises:

- (a) administering to said subject a composition containing a detectably labeled binding ligand capable of binding to ICAM-1, said ligand being selected from the group consisting of an antibody and a fragment of an antibody, said fragment being capable of binding to ICAM-1, and
- (b) detecting said binding ligand.

61. A method of diagnosing the presence and location of inflammation resulting from a response of the specific defense system in a mammalian subject suspected of having said inflammation which comprises:

- (a) incubating a sample of tissue of said subject with a composition containing a detectably labeled binding ligand capable of identifying a cell which expresses ICAM-1, and
- (b) detecting said binding ligand.

62. The method of any one of claims 60 or 61, wherein said binding ligand is bound to ICAM-1 present in said sample of tissue.

63. The binding ligand of any one of claims 60 or 61, wherein said binding ligand is selected from the group consisting of: a monoclonal antibody capable of binding to ICAM-1; and a fragment of said monoclonal antibody, said fragment being capable of binding to ICAM-1.

64. The binding ligand of claim 63, wherein said monoclonal antibody is the monoclonal antibody R6-5-D6.

65. A method of diagnosing the presence and location of a tumor cell which expresses a member of the LFA-1 family of molecules in a subject suspected of having such a cell, which comprises:

(a) administering to said subject a composition containing a detectably labeled binding ligand capable of binding to a member of the LFA-1 family of molecules, said ligand being selected from the group consisting of ICAM-1 and a functional derivative of ICAM-1 and

(b) detecting said binding ligand.

66. A method of diagnosing the presence and location of a tumor cell which expresses a member of the LFA-1 family of molecules in a subject suspected of having such a cell, which comprises:

(a) incubating a sample of tissue of said subject in the presence of a composition containing a detectably labeled binding ligand capable of binding to a member of the LFA-1 family of molecules, said ligand being selected from the group consisting of ICAM-1 and a functional derivative of ICAM-1 and

(b) detecting said binding ligand which is bound to a member of the LFA-1 family of molecules present in said sample of tissue.

67. The functional derivative of claim 4 wherein said fragment contains domains 1, 2 and 3 of ICAM-1.

68. The functional derivative of claim 4 wherein said fragment contains domains 1 and 2 of ICAM-1.

69. The functional derivative of claim 4 wherein said fragment contains domain 1 of ICAM-1.

70. The method of claim 30 wherein said fragment contains domains 1, 2 and 3 of ICAM-1.

71. The method of claim 30 wherein said fragment contains domains 1 and 2 of ICAM-1.

72. The method of claim 30 wherein said fragment contains domain 1 of ICAM-1.

73. A method for treating inflammation resulting from a response of the non-specific defense system in a mammalian subject which comprises providing to a subject in need of such treatment an amount of an anti-inflammatory agent sufficient to suppress said inflammation; wherein said anti-inflammatory agent is selected from the group consisting of: a fragment of ICAM-1 containing domains 1, 2 and 3 of ICAM-1; a fragment of ICAM-1 containing domains 1 and 2 of ICAM-1; and a fragment of ICAM-1 containing domain 1 of ICAM-1.

74. A method for treating inflammation resulting from asthma which comprises providing to a subject in need of such treatment an amount of an anti-inflammatory agent sufficient to suppress said inflammation; wherein said anti-inflammatory agent is selected from the group consisting of: an antibody capable of binding to ICAM-1; a fragment of an antibody, said fragment being capable of binding to ICAM-1; ICAM-1; a functional derivative of ICAM-1; and a non-immunoglobulin antagonist of ICAM-1.